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# USE OF MODIFIED CAMP TEST FOR PRELIMINARY NONSEROLOGIC IDENTIFICATION OF *VIBRIO CHOLERAE* IN STOOL SPECIMENS

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*ABSTRAK*

***PENGGUNAAN CAMP TEST UNTUK IDENTIFIKASI NON SEROLOGIK VIBRIO CHOLERAE DALAM TINJA***

*Suatu modifikasi uji CAMP digunakan bersama dengan reaksi biokimiawi untuk identifikasi* Vibrio cholerae *pada sampel klinis. Dari 579 usap dubur penderita diare, 92 (16%) memberikan hasil isolasi* V. cholerae *01 biotipe El Tor dan 34 (6%)* V cholerae *non-01. Semua isolat* V. cholerae *01 El Tor menunjukkan reaksi CAMP positif kuat dengan gambaran hemolisis sinergistik lengkap berbentuk sosis; sedangkan* V. cholerae *non-01 memberikan reaksi CAMP yang sempit dengan pola hemolisis menyerupai bulan sabit. Hasil uji CAMP yang dilakukan bersama dengan reaksi biokimiawi sesuai dengan metode biakan konvensional yang menyertakan tes aglutinasi dengan antiserum* V. cholerae *01 untuk mengidentifikasi* V. cholerae.

*Key words:* Vibrio cholerae *identification, CAMP test.*

## INTRODUCTION

Cholera continues to be a devastating disease of immense global significance and a major public health problem in developing countries. *Vibrio cholerae* 01, the etiologic agent of cholera, causes acute diarrhea, leading to rapid and potentially fatal fluid and electrolytes loss. Since the early 1960s the seventh pandemic has spread from Southeast Asia across the Middle East and into Africa and Central America [1] The recent introduction of cholera into South America [2] has reemphasized the need to recognize *V cholerae* 01 as early as possible in the level of a minimal equipped laboratory

In many developing countries, simpler identification schemes are employed for identification of *V. cholerae*. One such scheme involves inoculating suspected *V. cholerae* colonies from the isolation plate, usually a thiosulfate citrate bile salts sucrose (TCBS) onto Kligler's iron agar (KIA) medium. Cultures yielding an alkaline slant over acid butt with no gas or $H_2S$ are then tested for oxidase activity and ability to agglutinate in *V cholerae* 01 antiserum [3].

The key confirmation for identification of *V. cholerae* 01 is agglutination in specific antisera raised against the 01 antigen. Diagnostic antisera are produced commercially;

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unfortunately these antisera are expensive and cost prohibitive for many laboratories in the developing countries. In addition, these antisera are not always readily available, it may take several weeks or months to obtain them.

Recently, our laboratory reported [4] the use of a CAMP (Christie, Atkins, and Munch-Petersen) test for biogrouping *V. cholerae* 01 strains and to distinguish them from strains of *V. cholerae* non-01. The test utilizes synergistic hemolysis in the presence of *Staphylococcus aureus* beta-lysin (toxin) and a CAMP-like factor of *V. cholerae*. The size of synergistic hemolytic zone correlates with the biogroup of the *V. cholerae* isolate. In our present study, using a combination of simple biochemical tests, namely reactions in KIA, motility indole ornithine (MIO), oxidase test, and the modified CAMP technique without employing *V cholerae* 01 specific antisera for the agglutination test, we were able to identify all *V. cholerae* isolates which were identified by the conventional culture techniques using 01 agglutination test.

## MATERIALS AND METHODS

### Specimen collection and culture procedure

Rectal swabs were collected from patients with watery diarrhea during January through March 1995 and were processed according to the conventional culture procedures [5] Briefly, two swabs were obtained from the patients, placed in the Cary Blair medium and transported to NAMRU-2 laboratory Upon arrival, the swabs were directly cultured onto thiosulfate citrate bile salts sucrose (TCBS) (Difco Laboratories, Detroit, MI.) agar and the plates were then incubated aerobically 36±1°C for 18-20 h. Yellow, flat, translucent colonies on TCBS which were typical for *V cholerae* were picked (minimally 2 colonies) using an inoculating needle and transferred into KIA and MIO. Oxidase test was performed on cultures from the KIA slants. Cultures that were oxidase positive, and demonstrated an alkaline slant over acid butt and no gas or $H_2S$ in KIA, and were motile, ornithine positive, and indole positive were presumptively identified as *V cholerae*. They were then confirmed by the slide agglutination method with *V. cholerae* polyvalent 01 and type-specific Ogawa and Inaba antisera. A clear-cut agglutination within 30 seconds confirmed the final identity.

### Modified CAMP test

Growth from KIA was used for the CAMP test. Blood agar medium was prepared from tryptic soy agar plus 5% washed (three times in normal saline) defibrinated sheep erythrocytes. The medium was poured into disposable petri dishes to a depth of approximately 3 mm. Plates were stored at 2-8°C until use. Prior to the test, blood agar plates were warmed at 37°C for 30 min. The CAMP test was peformed according to the method described previously[4]. Briefly, the bottom of a blood agar plate was divided into quadrants using a marking pen; and each quadrant was again divided into smaller quadrants. Using a bacteriological needle, beta-toxin producing *S. aureus* was stabbed inoculated into the blood agar medium at the center of each cross of the quadrant. *V. cholerae* isolates to be CAMP-tested were then stab-inoculated in the same manner at each of the four points on the cross at a distance of approximately 9 mm from the *S. aureus* stab. A total of 16-20 isolates could be tested on one blood agar plate with this method. The plates were placed in a candle jar and incubated at 36±1°C for 18 h. Test plates were immediately interpreted following 18 h incubation.
The CAMP reactions for *V. cholerae* 01 biotype El Tor appear as sausage-shaped zones of complete synergistic hemolysis, whereas those

of *V. cholerae* non-01 strains appear as narrow, thin hemolytic zones of crescent-shape. Strains of *V. cholerae* 01 bioytype classical are CAMP-negative [4].

## RESULTS

Out of a total of 579 rectal swabs which were processed by the conventional culture method, 92 (16%) specimens yielded *V. cholerae* 01 biotype El Tor and 34 (6%) were *V. cholerae* non-01. Serotyping of the *V. cholerae* 01 isolates with specific 01 antiserum detected 90 Ogawa and 2 Inaba serotypes. Whereas all 34 strains of *V. cholerae* non-01 which showed an identical pattern of biochemical reactions to those of *V. cholerae* 01, did not agglutinate in polyvalent 01 antiserum.

When CAMP-tested, all 92 cultures identified as *V. cholerae* 01 biotype El Tor by the conventional culture procedure employing serological typing method, demonstrated large sausage-shaped zones of complete synergistic hemolysis (CAMP positive). The 34 conventionally identified *V. cholerae* non-01 were also CAMP positive but their zones of synergistic hemolysis were narrow and crescent-shaped. Typical CAMP reaction patterns for *V. cholerae* 01 and non-01 are shown on Fig. 1

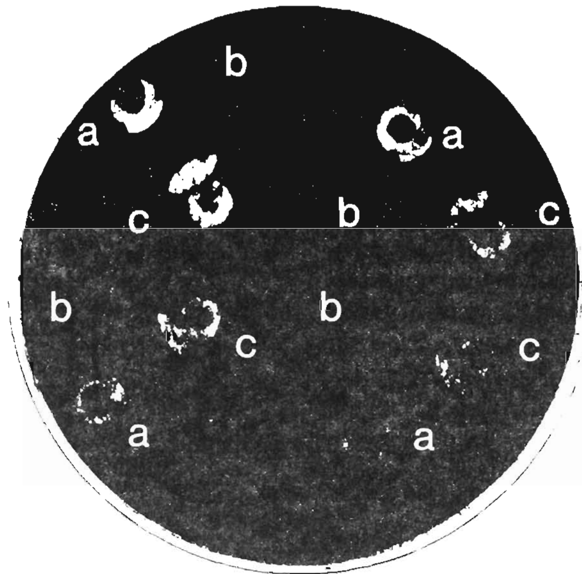


**Fig. 1. Typical CAMP reactions for *V. cholerae* 01 and non-01. Crescent-shaped CAMP positive *V. cholerae* non-01 (a), CAMP negative *V. cholerae* classical (b), and sausage-shaped CAMP positive *V. cholerae* El Tor (c).**

Fifty-one cultures which were picked from the TCBS plates and biochemically tested and identified as non-cholera vibrios were also negative by the CAMP test.

## DISCUSSION

There was 100% agreement between the CAMP test and the conventional culture methods for the identification of *V. cholerae* 01 and non-01. All isolates of conventionally identified *V. cholerae* 01 biotype El Tor demonstrated a typical CAMP reaction which appeared as a sausage-shaped zone of complete synergistic hemolysis on blood agar plate, whereas *V. cholerae* non-01 isolates which were also CAMP positive, showed narrow synergistic hemolysis of crescent-shape. The difference in the CAMP reactivity between the El Tor and non-01 strains is quantitative rather than qualitative.

Although classical strains have never been found in Indonesia, it is still necessary to further characterize *V. cholerae* 01 isolated. In this case, a common practice for most laboratories is to include polymyxin B susceptibility test in the culture method for differentiating the classical and the El Tor strains [3]. Findings reported previously [4] showed that the CAMP test could be used to distinguish between the classical and the El Tor biotype of *V cholerae* 01 strains; the classical strains were CAMP negative and these observations were in agreement with the results of polymyxin B susceptibility test.
In this study, we detected no classical strains of *V cholerae* 01 The 51 cultures with negative CAMP reactions were not *V cholerae* Although they were nonhemolytic and their CAMP reactions were negative like those of the classical strains, their biochemical characteristics were not typical of *V. cholerae*.

Agglutination in *V. cholerae* 01 polyvalent antiserum is sufficient to provide identification of *V. cholerae* 01. However, further characterization into Ogawa and Inaba serotypes is necessary for the purpose of epidemiology. The major surface antigen employed in characterization of *V. cholerae* is the O antigen. Flagellar (H) antigen is also present, but the value of this antigen for species identification is limited because of the presence of common H antigen among all *vibrio* species [5]. Unlike other enteric species for which the term serotype usually signifies an assortment of O and H antigens, for *V cholerae* it usually refers to different antigenic forms of the 01 antigen. The 01 serogroup is divided into three antigenic forms called Ogawa, Inaba, and Hikojima. These antigenic forms are referred to as serotypes or subtypes. The O antigen of *V cholerae* 01 consists of 3 factors designated A, B, and C [6] The difference among the subtypes is largely quantitative; Ogawa strains produce the A and B antigens and a small amount of C, while Inaba strains produce only the A and C antigens [7] Specific Ogawa and Inaba sera are prepared by absorption with the other subtype. The Hikojima subtype is rare and unstable and is not recognized by some authorities [3]

Traditionally, culture which shows biochemical reactions of *V cholerae* is then tested for its agglutination in polyvalent 01 antiserum. This antiserum is raised from rabbit and consist of a mixture of anti-ABC factors. Isolates that agglutinate in 01 polyvalent antiserum is further tested with monovalent Ogawa and Inaba antisera. Although commercially available, *V cholerae* antisera are not readily obtained in most developing countries. Purchasing them from sources outside the countries requires 2-3 or more months which leads local laboratories to raise their own

*Vibrio* antisera or use local products. Unfortunately, most of these antisera are not standardized.
Usually, commercial antisera are satisfactory, but in a report by Donovan and Furniss [8], two laboratories using commercial antisera misidentified *V. cholerae* non-O1 as O1.
It is recommended to perform the slide agglutination test by using growth from the KIA. However, some laboratories do the test by using growth directly from TCBS, which in many instances causes false interpretation due to poor emulsification of the colonies [9]. When growth from TCBS is used for the test, it will agglutinate slowly or weakly; or it will form a particulate, non-smooth suspension which may be interpreted as an agglutination.

The advantage of the CAMP test for *V. cholerae* identification is that individual colonies can be tested directly from TCBS plate as well as growth from the KIA. Additionally, the cost of the antiserum for one test is approximately 15 to 20 times higher than that of the CAMP test. In the CAMP method, one blood agar plate can be used to test 16-20 individual cultures or colonies.

It should be emphasized, though, that because of the quantitative nature of the CAMP reaction, this test requires a standardized approach. The test plates should be read immediately following 18-20 h of incubation as longer period of incubation may cause a difficulty in the interpretation of the reaction [10]. Maintaining the distances between stabs of *V. cholerae* and *S. aureus* is also necessary to obtain the best results as they influence the CAMP reaction [4].

The findings we report show our simple, non-serologic method for the preliminary identification of *V. cholerae* isolates to be a reliable screening tool.

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